

## **CLAIMS**

What is claimed is:

1. A computer-implemented method for segmenting objects, the method comprising the steps:

reading an image dataset containing an electronic representation of an image, said image having a plurality of data points;

determining one or more initial defining sets by finding interconnected sets of said data points;

determining one or more valid defining sets by applying one or more restricting conditions to said initial defining sets; and

identifying one or more segmented objects.

2. The computer-implemented method of claim 1, wherein said data points have an associated intensity value.
3. The computer-implemented method of claim 1, wherein said data points have an associated Red, Green, Blue value.
4. The computer-implemented method of claim 1, wherein said data points are pixels.
5. The computer-implemented method of claim 1, wherein said data points are voxels.
6. The computer-implemented method of claim 1, wherein finding interconnected sets of said data points includes finding a path of successive neighboring data points where a subsequent neighboring data point has an intensity value equal to or greater than an intensity value of a previous data point; and  
  
wherein said path is limited to a predetermined length of data points.
7. The computer-implemented method of claim 1, wherein the path is linear.
8. The computer-implemented method of claim 7, wherein the path is also diagonal.

9. The computer-implemented method of claim 1, wherein said electronic representation of an image is a 2-dimensional representation.
10. The computer-implemented method of claim 1, wherein said electronic representation of an image is a 3-dimensional representation.
11. The computer-implemented method of claim 1, wherein said electronic representation of an image is a grey-scale or color representation.
12. The computer-implemented method of claim 1, wherein said image dataset is in JPEG, BMP, TIFF or GIF format.
13. The computer-implemented method of claim 1, wherein said image dataset is a database, a computer file, or an array of data in computer memory.
14. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes applying a criteria for an initial defining set, such that said initial defining set will be excluded from being a valid defining set.
15. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes applying a criteria for an initial defining set, such that said initial defining set will be included as being a valid defining set.
16. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes excluding said initial defining set where the initial defining set has a volume greater than or equal to a predetermined maximum volume.
17. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes excluding said initial defining set where the initial defining set has a volume lesser than or equal to a predetermined minimum volume.
18. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes excluding an initial defining set where the initial defining set has an extent in an x- and y- direction greater than a predetermined maximum extent.
19. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes excluding an initial defining set where the initial defining set

has an extent in a z- direction greater than a predetermined maximum extent.

20. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes excluding said initial defining set where the initial defining set has a sphericity greater than or equal to a predetermined maximum sphericity.

21. The computer-implemented method of claim 1, wherein applying one or more restricting conditions includes excluding said initial defining set where the initial defining set has a sphericity lesser than or equal to a predetermined minimum sphericity.

22. The computer-implemented method of claim 1, further comprising counting said segmented objects.

23. The computer-implemented method of claim 1, further comprising displaying said segmented objects in a graphical user interface.

24. The computer-implemented method of claim 1, further comprising determining a centroid for said segmented objects.

25. The computer-implemented method of claim 24, further comprising displaying said centroids for said segmented objects.

26. The computer-implemented method of claim 25, further comprising overlaying a grid with said centroids to aid in visual reference of said centroids.

27. The computer-implemented method of claim 1, further comprising determining an intensity threshold for said segmented objects.

28. The computer-implemented method of claim 27, wherein the intensity threshold for a particular segmented object is the intensity of the dimmest voxel, where said image is a 3-dimensional image.

29. The computer-implemented method of claim 27, wherein the intensity threshold for a particular segmented object is the intensity of the dimmest pixel, where said image is a 2-dimensional image

30. The computer-implemented method of claim 1, further comprising smoothing said image to remove image artifacts from said image before the step of determining one or more

initial defining sets.

31. A method of determining transcriptional activity of a gene of interest comprising the steps of:

obtaining a biological sample;

imaging the sample to obtain an image dataset; and

using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the transcriptional activity.

32. A method of diagnosing a hyperproliferative disease comprising the steps of:

obtaining a biological sample from a subject;

imaging the sample to obtain an image dataset; and

using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the hyperproliferative disease.

33. The method of claim 32, wherein the hyperproliferative disease is further defined as cancer.

34. The method of claim 34, wherein the cancer comprises a neoplasm.

35. The method of claim 34, wherein the neoplasm is selected from the group consisting of melanoma, non-small cell lung, small-cell lung, lung hepatocarcinoma, retinoblastoma, astrocytoma, glioblastoma, leukemia, neuroblastoma, squamous cell, head, neck, gum, tongue, breast, pancreatic, prostate, renal, bone, testicular, ovarian, mesothelioma, sarcoma, cervical, gastrointestinal, lymphoma, brain, colon, and bladder.

36. The method of claim 35, wherein the neoplasm is prostate cancer.

37. A method of screening a subject at risk for developing a hyperproliferative disease comprising the steps of:

obtaining a biological sample from a subject;

imaging the sample to obtain an image dataset; and

using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the hyperproliferative disease.

38. A method of staging or monitoring a hyperproliferative disease in a subject comprising the steps of:

obtaining a biological sample from a subject;

imaging the sample to obtain an image dataset; and

using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the hyperproliferative disease.

39. A method of determining the expression level of a gene of interest comprising the steps of:

obtaining a biological sample;

imaging the sample to obtain an image dataset; and

using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the expression level of the gene.

40. A method of determining the expression level of a protein of interest comprising the steps of:

obtaining a biological sample;

imaging the sample to obtain an image dataset; and

using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the expression level

of the protein.

41. A method of determining a cell structure of interest comprising the steps of:
- obtaining a biological sample;
  - imaging the sample to obtain an image dataset; and
  - using the computer-implemented method of claim 1 to identify one or more segmented objects, wherein the identified segmented objects correlate to the cell structure.